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Original article

## Dissolved heavy metal determination and ecotoxicological assessment: a case study of the Corumbataí River (São Paulo, Brazil)

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The aim of this one-year study (August 2009 to July 2010) was to evaluate the Corumbataí River water polluted by anthropogenic sources and see how it affects the reproduction of the microcrustacean *Ceriodaphnia dubia* (Richard, 1984) in laboratory conditions over seven days of exposure to water samples collected monthly at six different locations. We determined the concentrations of zinc (Zn), copper (Cu), nickel (Ni), lead (Pb), and cadmium (Cd), as well as physicochemical parameters such as dissolved oxygen, conductivity, water temperature, and pH. Dissolved oxygen and conductivity demonstrated anthropogenic influence, as dissolved oxygen concentration decreased and conductivity increased from the upstream to the downstream stretch of the river. The effects on *C. dubia* were observed in the months with high precipitation, but the toxicity cannot be associated with any particular contaminant. Heavy metal levels kept well below the limit values. Zn and Pb had the highest concentrations in the water during the sampling period, probably due to the industrial and agricultural influence. However, these levels do not seem to be associated with precipitation, which suggests that their primary source was industry. Physicochemical parameters, the ecotoxicological assay, and determination of heavy metals proved to be efficient tools to evaluate aquatic environments.

**KEY WORDS:** *Ceriodaphnia dubia*; inorganic contaminants; physicochemical parameters; toxicity test; water

Rivers are often polluted by indiscriminate disposal of sewage and industrial wastes and a plethora of human activities. Preservation of river water quality requires effective monitoring, but the assessment of aquatic environment contamination is often limited to physicochemical analyses (1). According to Forget et al. (2) and Cairns (3), this is not enough to determine the real state of water quality and the analysis should be complemented with ecotoxicological assays that look into the interaction between the living organisms and the concentrations of chemical agents in the

aquatic environment. For this purpose, ecotoxicological assays often use sensitive organisms such as microcrustaceans.

In addition to physicochemical parameters and ecotoxicological assays, water monitoring should include the determination and quantification of metals (4-6). Although their impact has no visible influence compared to other pollutants, heavy metals can cause long-term effects on ecosystems (7) due to their persistence, bioaccumulation, and biomagnification in the food chain (8).

The aim of our study was to evaluate the water quality of the Corumbataí River by combining these three monitoring methods over a one-year period.

The Corumbataí River basin is an important water source located in the state of São Paulo, Brazil, between latitude 22°05'–22°30'S and longitude 47°30'–47°50'W, and includes an area of approximately 1.690 km<sup>2</sup> (9). This river was chosen for this study because it is located in one of the most industrialised regions of São Paulo and receives effluents from industries such as textile, ethanol, and chemistry. In addition, this is one of the largest sugarcane-producing regions of Brazil (10), and according to Wahlberg et al. (11) agricultural areas may also contribute to heavy metal contamination of aquatic environments.

Earlier studies conducted in the Corumbataí River to characterise its water quality have used different methodologies, but never a combination that would involve long-term ecotoxicological assays and heavy metal determination. In one of these studies, Jardim et al. (12) established acute and chronic toxicity of the Corumbataí River water and sediment to the microcrustaceans *Daphnia magna* (Straus, 1820) and *Daphnia similis* (Claus, 1876) in two sampling periods over less than a year. In another study, Cetra and Petrere (9) observed that species richness was the highest in locations with greater vegetation cover and preserved riparian forest.

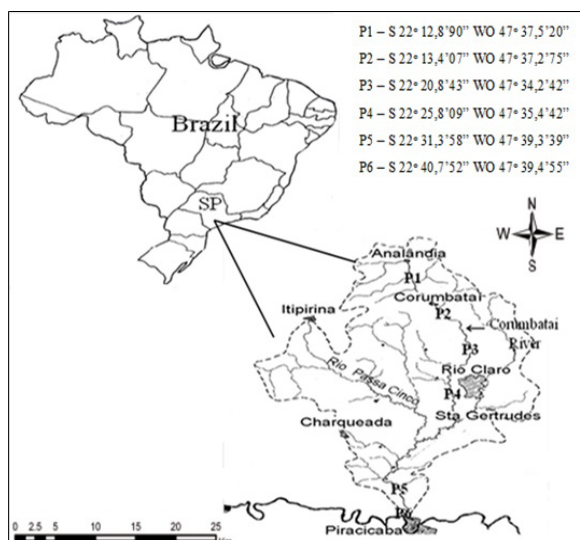
The only study (by Pescim et al., 13) determining metals in the Corumbataí River reinforces the need for further investigation into the influence of industrial and agricultural effluents on this ecosystem.

## MATERIALS AND METHODS

### Sampling sites and physicochemical parameters

Surface water samples were collected from six locations along the centre of the Corumbataí River course on a monthly basis from August 2009 to July 2010 (see Figure 1).

Physicochemical parameters dissolved oxygen and water temperature, were measured at the river using a YSI Model 55 (12-foot cable length) handheld oxymeter (YSI, Yellow Springs, OH, USA). Conductivity (conductivimeter Ação Científica MPA-150 P, Marconi, Piracicaba, Brazil) and pH (pH meter Ação Científica MPA-210 P) were determined in the



**Figure 1** Water sampling points (P) (n=72) along the Corumbataí River, São Paulo, Brazil

P1 and P2: upstream and downstream of the Corumbataí City; P3 and P4: upstream and downstream of the city of Rio Claro; P5 and P6: upstream of and in Piracicaba, respectively

laboratory immediately after sampling. The sampling followed the Standard Methods of Examination of Water and Wastewater of the American Public Health Association (14). The results of physicochemical parameters are expressed as the mean±standard deviation of all measurements over the sampling period, including the range and the coefficient of variation.

### Precipitation

Precipitation data (rainfall accumulation) in the Corumbataí River for the study period was provided by the Water and Electric Energy Department (DAEE) of Piracicaba (São Paulo, Brazil). Precipitation was measured monthly at four monitoring locations in the cities of Analândia, Corumbataí, Rio Claro, and Piracicaba.

### Culturing and toxicity testing with *Ceriodaphnia dubia*

*Ceriodaphnia dubia* was used in this study because it has been standardised for ecotoxicological studies by the Brazilian Technical Standard Association (ABNT) (15) and is readily available and easy to cultivate in a laboratory. All procedures for culturing and toxicity testing followed the ABNT NBR 13373 standard (15). The cultures (reconstituted water) were maintained in the Laboratory of Aquatic Ecotoxicology, Centre for Nuclear Energy in Agriculture (Piracicaba, São Paulo, Brazil) in an incubator (40 organisms in

each culture) at  $25 \pm 2$  °C and 16:8 h light/dark cycle. Reconstituted water was prepared using 18 MΩ deionised water and reagent-grade chemicals. The pH ranged from 7 to 7.6 and hardness from 48 to 50 mg L<sup>-1</sup> of CaCO<sub>3</sub>, which was in accordance with the ABNT recommendations. The culture medium was renewed twice a week, and the *C. dubia* was fed with algae *Pseudokirchneriella subcapitata* (Hindak, 1990) three times a week ( $1 \times 10^5$  cells per organism). Once a month, the test organisms were evaluated for their sensitivity (lethality) using NaCl as a reference substance (15). The 48-hour median lethal concentration (LC<sub>50</sub>) of NaCl to *C. dubia* was determined using the Trimmed Spearman-Kärber method (16).

For the toxicity test, one individual of *C. dubia* (<24 h old) was housed in a 30-mL glass container with 20 mL of test solution (unfiltered and undiluted water sample). Ten replicates were used for each sampling point plus a control group (culture medium). The test was conducted under the same conditions as the culture maintenance. Water samples were kept in a refrigerator below -20 °C to maintain their characteristics. The test lasted seven days, and the solutions were renewed every two days with the adult microcrustacean transferred to the new solution. During the renewal, the organisms were fed with *P. subcapitata* ( $1 \times 10^5$  cells per organism), and the number of neonates produced was recorded as reproduction endpoint.

#### Metal determination

Water samples were homogenised, filtered through 0.45-µm syringe filters, and added to a 300-mL beaker.

They were then acidified with 4 mL of HNO<sub>3</sub> and evaporated on a heating plate to 10 mL. After the heating, the samples were maintained at reflux for 30 min, and then transferred to 50-mL volumetric flasks, and the volume was filled up with deionised water. Cd, Zn, Cu, Pb, and Ni were analysed using a flame atomic absorption spectrometer (AA 7000, Shimadzu, Columbia, MA, USA).

The limit of quantification and the limit of detection for each metal were determined following the guideline about the validation of analytical methods by the National Agency for Sanitary Vigilance (ANVISA) (17).

#### Statistical analysis

To compare the mean values of *C. dubia* reproduction between the control group and the groups treated with the Corumbataí River water we used one-way analysis of variance (ANOVA) followed by Tukey's test. Data were analysed using SAS software version 9.2 (SAS Institute Inc, Cary, NC, USA).

## RESULTS AND DISCUSSION

#### Precipitation

Figure 2 shows the Corumbataí River precipitation over the sampling period. High precipitation was recorded in November 2009 (244.00 mm) for Analândia and December 2009 (375.90 mm), January 2010 (582.90 mm), and February 2010 (238.90 mm) for Rio Claro.

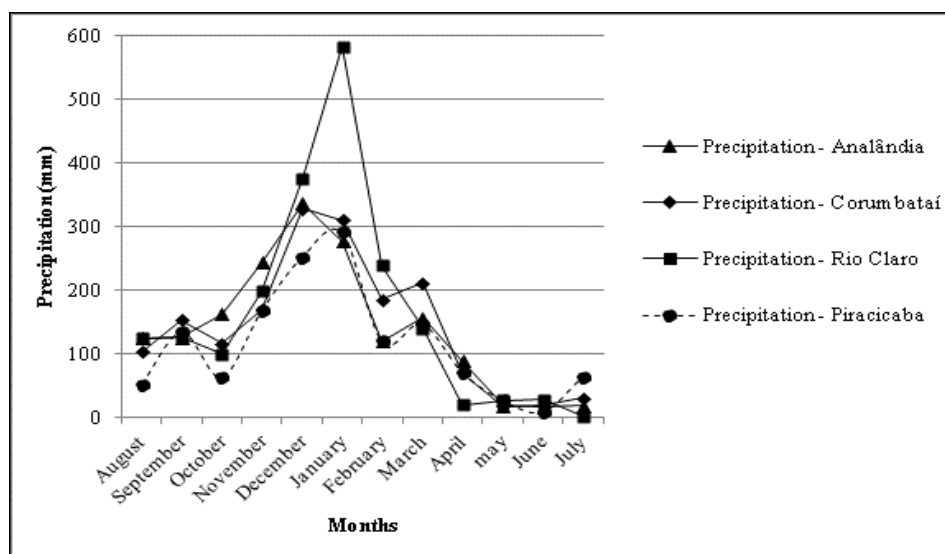


Figure 2 Total precipitation (mm) in the Corumbataí River during the sampling period

*Physicochemical parameters*

The physicochemical features of sampled water are presented in Table 1. Mean water temperature varied between 19.37 °C and 21.74 °C. Water temperature increased from Point 1 to Point 6 (downstream) (Table 1), probably due to the heat produced by sunlight because the water samples were collected in the morning starting at Point 1 and ending in the afternoon at Point 6.

For its biota to be preserved, the Brazilian rivers should have a pH ranging from 6 to 9, according to CONAMA (18). In our study, the mean pH ranged from 7.21 to 7.51 (Table 1), which is within this range.

In contrast, according to CONAMA (18), dissolved oxygen should not drop below 5.0 mg L<sup>-1</sup>, but the mean values of dissolved oxygen at all sampling points were below this limit (Table 1). They decreased from the upstream to the downstream of the river, probably due to water temperature increase, as mentioned above. Domestic sewage from Rio Claro and Piracicaba may have also contributed its drop.

The highest conductivity values were observed at Points 3, 4, 5, and 6 (Table 1). High conductivity is expected in samples collected in areas affected by industrial and agricultural activities that release dissolved ions through effluents and soil leachates. Accordingly, only Points 1 and 2 were not affected by industrial activities.

*Chronic toxicity test with C. dubia*

The mean, 48-hour LC<sub>50</sub> of NaCl to *C. dubia* for all months was 1.89±0.22 mg L<sup>-1</sup>, with the coefficient

of variation of 11.64 %. These values are within the variability limit (mean±2 standard deviation) and acceptable by the national standard (15).

Water was considered toxic to organisms when mean reproduction was significantly lower than control ( $p<0.05$ ). This was the case with water samples collected at nearly all sampling points from November 2009 to February 2010 (Table 2). Water collected from August to October 2009 and from March to July 2010 showed lower toxicity, as one or two points presented a significant difference compared to control ( $p<0.05$ ).

It is difficult to associate the cause of toxicity with a particular substance or element when organisms are exposed to river waters, because rivers contain complex mixtures of contaminants. In the case of the Corumbataí River, for example, many substances such as heavy metals have been detected in the water, sediments, and fish (19, 20). Beside heavy metals, several classes of herbicides have been reported (21). Botelho (22) reported atrazine and ametrine in the Piracicaba River, which make part of the same basin as the Corumbataí River. These two herbicides are often used in sugarcane crops in the region. Many authors demonstrated the toxicity of herbicides to aquatic organisms (23-28).

Our chronic toxicity findings seem to be associated with run-offs carrying contaminants such as pesticides from agricultural areas, as indicated by precipitation rates. The samples that had higher toxicity were collected in the months with higher precipitation. The exception is March, which showed toxicity at two

**Table 1** Physicochemical parameters measured at the sampling points from August 2009 to July 2010

Points		Water temperature (°C)	pH	Conductivity (µs cm <sup>-1</sup> )	Dissolved oxygen (mg L <sup>-1</sup> )
1	Mean±SD	19.37±3.43	7.51±0.51	44.66±7.68	4.70±1.19
	Range	12.40-23.50	6.70-8.61	34.30-58.20	2.82-7.21
	Coefficient of variation	17.71	6.79	17.20	25.32
2	Mean±SD	19.73±3.45	7.44±0.64	40.30±6.74	4.67±1.18
	Range	12.90-23.80	6.43-8.66	30.10-46.90	2.49-6.86
	Coefficient of variation	17.49	8.60	16.72	25.27
3	Mean±SD	21.14±3.50	7.22±0.47	56.48±12.41	4.04±1.21
	Range	14.10-23.80	6.44-8.06	37.80-71.60	2.50-6.16
	Coefficient of variation	16.56	6.51	21.97	29.95
4	Mean±SD	21.41±3.76	7.21±0.48	89.48±19.78	3.49±0.90
	Range	14.10-25.70	6.23-7.88	72.50-116.00	2.11-5.24
	Coefficient of variation	17.42	6.66	22.10	25.78
5	Mean±SD	21.41±4.02	7.21±0.47	89.48±52.76	3.64±1.11
	Range	14.70-26.90	6.65-8.11	52.60-233.10	2.23-6.02
	Coefficient of variation	18.78	6.52	58.96	30.50
6	Mean±SD	21.74±3.92	7.36±0.43	148.30±46.56	3.74±0.86
	Range	15.70-28.50	6.37-8.07	99.30-215.70	1.94-5.12
	Coefficient of variation	18.03	5.84	52.06	22.00



**Table 2** Mean values of *Ceriodaphnia dubia* reproduction (neonates/female) from August 2009 to July 2010 after exposure to the Corumbataí River water

Samples	Months											
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Control	19.70 <sup>a</sup>	21.70 <sup>a</sup>	21.70 <sup>a</sup>	21.10 <sup>a</sup>	22.80 <sup>a</sup>	24.50 <sup>a</sup>	23.00 <sup>a</sup>	21.40 <sup>a</sup>	22.70 <sup>a</sup>	23.50 <sup>a</sup>	21.30 <sup>a</sup>	18.80 <sup>b</sup>
Point 1	7.88 <sup>b*</sup>	13.00 <sup>ab</sup>	8.33 <sup>b*</sup>	5.57 <sup>b*</sup>	11.70 <sup>b*</sup>	13.50 <sup>b*</sup>	15.80 <sup>ab</sup>	11.30 <sup>b*</sup>	13.20 <sup>b*</sup>	11.85 <sup>b*</sup>	12.22 <sup>ab</sup>	7.42 <sup>c**</sup>
Point 2	11.00 <sup>ab</sup>	8.66 <sup>b*</sup>	10.90 <sup>b</sup>	6.37 <sup>b*</sup>	10.60 <sup>b*</sup>	10.20 <sup>b*</sup>	22.80 <sup>a</sup>	14.55 <sup>ab</sup>	18.44 <sup>ab</sup>	14.20 <sup>b*</sup>	13.75 <sup>ab</sup>	7.70 <sup>c**</sup>
Point 3	14.00 <sup>ab</sup>	14.50 <sup>ab</sup>	12.70 <sup>ab</sup>	13.22 <sup>b*</sup>	13.20 <sup>ab</sup>	10.66 <sup>b*</sup>	6.60 <sup>b*</sup>	14.33 <sup>ab</sup>	19.14 <sup>ab</sup>	16.55 <sup>ab</sup>	9.20 <sup>b*</sup>	24.90 <sup>ab</sup>
Point 4	16.44 <sup>ab</sup>	14.50 <sup>ab</sup>	14.90 <sup>ab</sup>	23.88 <sup>a</sup>	11.40 <sup>b*</sup>	11.33 <sup>b*</sup>	8.00 <sup>b*</sup>	20.40 <sup>ab</sup>	17.50 <sup>ab</sup>	18.11 <sup>ab</sup>	8.80 <sup>b*</sup>	33.10 <sup>ae</sup>
Point 5	13.60 <sup>ab</sup>	17.60 <sup>ab</sup>	11.11 <sup>ab</sup>	19.77 <sup>ab</sup>	20.30 <sup>ab</sup>	15.20 <sup>b*</sup>	2.71 <sup>b*</sup>	14.90 <sup>ab</sup>	19.50 <sup>ab</sup>	21.33 <sup>ab</sup>	16.50 <sup>ab</sup>	26.77 <sup>ab</sup>
Point 6	11.50 <sup>ab</sup>	10.90 <sup>b*</sup>	7.20 <sup>b*</sup>	15.40 <sup>ab</sup>	11.70 <sup>b*</sup>	13.70 <sup>b*</sup>	5.60 <sup>b*</sup>	4.22 <sup>b*</sup>	18.87 <sup>ab</sup>	26.33 <sup>ab</sup>	19.10 <sup>a</sup>	32.90 <sup>ae</sup>

Differing letters denote significant difference (Tukey's test;  $p < 0.05$ ).

\*toxic

sampling points only but had high precipitation (Tables 1 and 2).

During the rainy season, contamination of the aquatic environment increases due to the availability of contaminants and nutrients from agricultural soils. Affonso et al. (29) established high trophic state index (TSI) in a lake in Brazil during the rainy season and concluded that the run-off of nutrients into the lake may have caused the eutrophication. The same was observed by Alves et al. (30). Likewise, Armas et al. (21) observed higher levels of herbicides (atrazine, ametrine, simazine, glyphosate, and clomazone) in the Corumbataí River at the beginning of the rainy season, confirming that run-off is an important route of contamination of aquatic environments.

### Metal findings

The tremendous use of Pb, Zn, Ni, Cd, and Cu over the past few decades has resulted in an increased concentration in the aquatic systems (31, 32). Due to their role in carrying away municipal and industrial wastewater and run-off from agricultural land, rivers are among the most vulnerable water bodies to metal pollution (33).

The method we used for the detection of metals in the Corumbataí River water had the coefficient of correlation higher than 0.99 for all metals and therefore high sensitivity. Its limit of quantification and detection are presented in Table 3.

Spatial variations of metal concentrations during the sampling period are presented in Table 4. Mean concentrations over the study period were lower than those set by the Brazilian environmental legislation (18) and World Health Organization (34). However, we can not be sure whether the measured concentrations are toxic or not to the aquatic organisms living in this river, as we have not conducted metal-specific toxicity assays. The most common was Zn, followed by Pb, Cd, and Ni. Cu kept below the limit of detection,

probably due to the water filtration of organic matter to which Cu binds (Table 4).

Points 1 (upstream of Corumbataí City) and 2 (downstream of Corumbataí City) are considered the locations with a lower influence of industrial activities, as they are located upstream and downstream from a small city. Points 3 through 6 are located in an area with higher industrial influence close to or within big cities such as Rio Claro and Piracicaba. Points with the higher occurrence of metals (in decreasing order) were 6, 3, 2, 1, 4, and 5. Although Points 1 and 2 are not considered under industrial influence, higher heavy metal levels compared to Points 4 and 5 may point to run-off of from soils cultivated with sugarcane.

In any case, the occurrence of any of the metals cannot be attributed to any specific activity as the river runs through both industrial and agricultural areas. Zn had the highest concentrations at Point 3 followed by Points 1, 4, 5, 2, and 6. Cd was determined at a higher concentration at Point 6 followed by Points 2 and 3. Concentrations of Ni were higher at Points 4, 6, and 1, whereas Pb presented higher concentrations at Point 6 followed by Points 2, 5, and 1.

Pb and Zn are among the most common toxic pollutants in industrial wastewater. Pb is a normal constituent of the earth's crust and trace amounts naturally occur in soil and water. According to Yi et al. (35), Pb may also originate from metal processing,

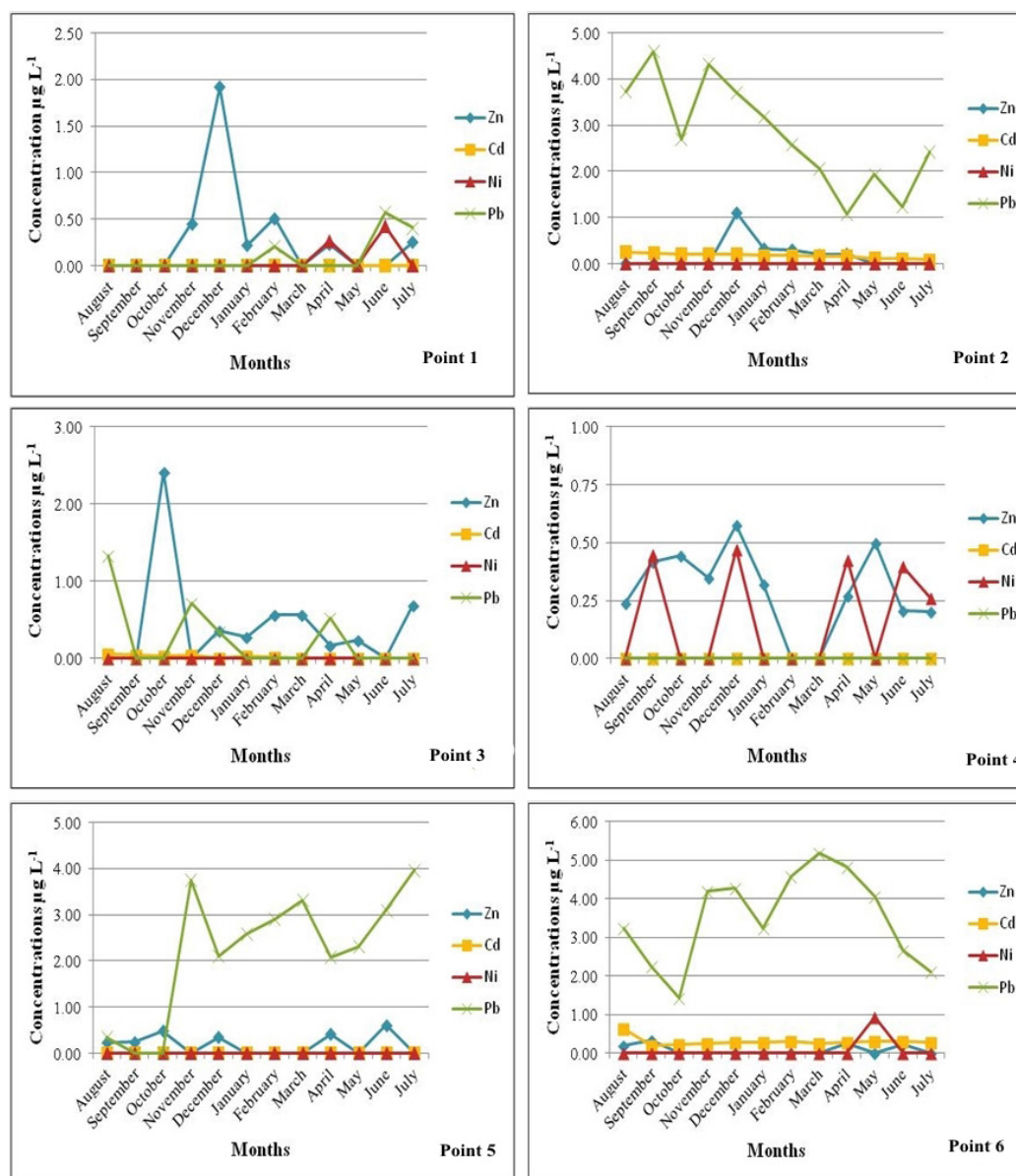
**Table 3** Limit of quantification ( $\mu\text{g L}^{-1}$ ) and limit of detection ( $\mu\text{g L}^{-1}$ ) for each of the metals analysed in the water samples of the Corumbataí River using flame atomic absorption spectrometry

Metals	Limit of quantification	Limit of detection
Zn	0.17	0.11
Cd	0.005	0.003
Cu	0.25	0.16
Pb	0.20	0.13
Ni	0.26	0.17

**Table 4** Mean metal concentrations ( $\mu\text{g L}^{-1}$ ) at each sampling point in the Corumbataí River from August 2009 to July 2010

Points	Metal means $\pm$ SD ( $\mu\text{g L}^{-1}$ )				
	Zn	Cd	Cu	Ni	Pb
1	0.30 $\pm$ 0.54	<LD	<LD	0.06 $\pm$ 0.14	0.10 $\pm$ 0.19
2	0.18 $\pm$ 0.32	0.17 $\pm$ 0.05	<LD	<LD	2.79 $\pm$ 1.14
3	0.44 $\pm$ 0.67	0.02 $\pm$ 0.02	<LD	<LD	0.24 $\pm$ 0.42
4	0.29 $\pm$ 0.18	<LD	<LD	0.17 $\pm$ 0.21	<LD
5	0.19 $\pm$ 0.23	<LD	<LD	<LD	2.20 $\pm$ 1.39
6	0.08 $\pm$ 0.13	0.30 $\pm$ 0.11	<LD	0.08 $\pm$ 0.27	3.51 $\pm$ 1.20
Maximally permitted value ( $\mu\text{g L}^{-1}$ )	180 <sup>a</sup> 10 <sup>b</sup>	1 <sup>a</sup> 3 <sup>b</sup>	9 <sup>a</sup> 2000 <sup>b</sup>	25 <sup>a</sup> 20 <sup>b</sup>	10 <sup>a</sup> 10 <sup>b</sup>

<LD = below the limit of detection. <sup>a</sup> and <sup>b</sup> refers to the maximally permitted value according to CONAMA (18) and WHO (34), respectively

**Figure 3** Temporal variations of Zn, Cd, Ni, and Pb at Points 1 through 6

electroplating industries, industrial wastewater, and domestic sewage. In addition to industrial effluents, the occurrence of Pb in our study can be associated with erosion and run-off with Pb-containing particles from soil cultivated with sugar cane.

Zn as zinc oxide is used in the ceramics industry, while zinc sulphate is common in textile industry and fertilisers, all of which are present in the Corumbataí River basin. Zn is toxic to very many aquatic species. For example, Zhu et al. (36) demonstrated that Zn significantly affects the development of *Gobiocypris rarus* (Ye and Fu, 1983) fish embryos, especially the development of body and heart after exposure from 0.01 to 1.000 mg L<sup>-1</sup>.

Figure 3 shows temporal variations of the elements at each sampling point. With the exception of Zn at Point 1, the results suggest that metal levels in the Corumbataí River are not related to precipitation (Figures 2 and 3). This reinforces our assumption that the contamination was mainly owed to industrial effluents as opposed to run-off. Many studies of the rivers of São Paulo have been developed to identify and quantify metals in different matrices. For example, Fostier et al. (20) observed that Hg contaminated all compartments (water, sediment, soil, and fish) in the Piracicaba River Basin. In the same basin França et al. (37) found more than 30 elements in suspended sediments, including those of great environmental importance such as Se, Sb, Cr, Mo, Zn, and Hg. In the study by Meche et al. (38), 11 of 14 metals (Al, As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, and Zn) were above the limit allowed by CONAMA (18). All these findings, including our own, confirm that all aquatic compartments are being contaminated. Considering that both population and industrial activity will increase heavy metals levels should be carefully monitored in the São Paulo Rivers.

## CONCLUSION

According to the physicochemical parameters (conductivity and dissolved oxygen) determined in our study, water quality decreased downstream of the river, probably due to the influence of industrial and domestic effluents. *C. dubia* turned out to be a sensitive organism for the evaluation of environmental contamination. The effects of the Corumbataí River water on the microcrustacean seem to be associated with precipitation, but the toxicity cannot be associated with any particular contaminant, and especially not

with the metals measured in this study, whose levels kept well below the limit values. Zn and Pb had the highest concentrations in the water during the sampling period probably due to the industrial and agricultural influence. However, these levels do not seem to be associated with precipitation, which suggests that their primary source was industry.

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## Conflict of interest

The authors declare no conflict of interest.

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**Sažetak****Određivanje otopljenih teških metala i ekotoksikološka procjena: prikaz istraživanja na rijeci Corumbataí (São Paulo, Brazil)**

Cilj ovog jednogodišnjeg istraživanja (od kolovoza 2009. do srpnja 2010.) bio je ocijeniti kakvoću rijeke Corumbataí, koja je onečišćena antropogenim izvorima, te ispitati kako ona utječe na razmnožavanje račića *Ceriodaphnia dubia* (Richard, 1984.) u laboratorijskim uvjetima sedmodnevne izloženosti uzorcima vode prikupljenima svakog mjeseca sa šest lokacija. Utvrđene su koncentracije cinka (Zn), bakra (Cu), nikla (Ni), olova (Pb) i kadmija (Cd) te fizikalnokemijskih parametara poput otopljenog kisika, provodljivosti, temperature vode i pH. Razine otopljenog kisika i provodljivost ukazali su na antropogene utjecaje, budući da se razina otopljenog kisika snižavala, a provodljivost rasla nizvodno. Toksično djelovanje na *C. dubia* zabilježeno je u uzorcima prikupljenima tijekom kišnih mjeseci, ali se ona ne može povezati s pojedinačnim zagađivačima. Razine teških metala bile su daleko ispod propisanih gornjih granica. Najviše koncentracije u vodi izmjerene su za Zn i Pb, što je vjerojatno povezano s industrijskom i poljoprivrednom aktivnosti. Te se razine međutim ne daju povezati s padalinama, što upućuje na zaključak da je njihov glavni izvor industrija. Fizikalnokemijski parametri, ekotoksikološki test i određivanje teških metala pokazali su se korisnim alatima za procjenu onečišćenosti vodenog okoliša.

**KLJUČNE RIJEČI:** *anorganska zagađivala; Ceriodaphnia dubia; fizikalnokemijski parametri; test toksičnosti; voda*

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